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TITLE: Development of a Novel Treatment for Food Allergy using a New Genetically Defined Mouse Model of the Disease

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14. ABSTRACT Loeys Dietz Syndrome (LDS) is an autosomal dominant disorder caused by mutations in the genes encoding the receptor for TG, a multifunctional cytokine that plays a key role in the development of mucosal tolerance. LDS patients are strongly predisposed to develop nearly all forms of allergic disease. The goals of this proposal are to examine whether LDS mice are more susceptible to developing peanut allergy, and whether treatment with losartan, an angiotensin II (ATII) receptor blocker that inhibits TGFbeta signaling, reduces the development and/or severity of allergic disease in this mouse model. Our experiments suggest that LDS mice do exhibit more severe symptoms of anaphylaxis, using both a passive systemic anaphylaxis model as well as a murine model of peanut allergy. These data support a role for dysregulated TGFbeta signaling in the development of food-induced anaphylaxis, suggesting that targeting this pathway with pharmacologic agents may have therapeutic benefit.					
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INTRODUCTION

Both food allergy (FA) and eosinophilic esophagitis (EE), a related condition characterized by eosinophilic inflammation in the esophagus and epithelial cell hyperplasia, have strong familial associations suggesting a relatively large genetic component. However, few candidate genes have been conclusively linked to the development of these disorders, frustrating efforts to develop mechanism-based therapies. Loeys-Dietz Syndrome (LDS), a recently described autosomal dominant disorder caused by mutations in either of the two genes encoding subunits of the TGF β receptor (TGFB1 or TGFB2), is the first Mendelian disorder to be specifically associated with the development of FA and EE. A mouse model of LDS strongly recapitulates the human phenotype, suggesting that altered TGF β signaling is sufficient to predispose to FA and EE, and that this pathway is therefore an attractive therapeutic target. In this proposal, we will test whether LDS mice are more susceptible to developing peanut allergy, and whether allergic disease in LDS mice can be prevented or improved by treatment with losartan. Losartan is an FDA-approved drug that inhibits the angiotensin II type I (AT-1) receptor, decreases TGF β signaling, and thereby targets the basic pathophysiologic defect caused by LDS mutations.

BODY

TASK 1: Demonstrate a critical role for altered TGF β signaling in the pathogenesis of food allergy by investigating the propensity of mice harboring LDS mutations to develop food allergy.

As proposed, we have undertaken experiments to determine whether LDS mice are more susceptible to developing peanut allergy using methods that have been described previously.¹ LDS mice and wild type (WT) littermates were sensitized with 1mg peanut protein and 10ug of cholera toxin by oral gavage four times (one week apart), as preliminary experiments demonstrated that 4 rounds of sensitization led to optimal anaphylactic responses (see 2012 progress report). Mice were then challenged intraperitoneally with peanut protein two weeks after the last sensitization dose was administered, and the severity of anaphylaxis (as measured by a drop in body temperature) was measured following peanut challenge. During the course of our preliminary experiments, we noticed that the esophagus of the LDS mice would often be impacted with food, presumably due to the dilatation and tortuosity of their esophagus as a result of their propensity to develop eosinophilic esophagitis. We suspected that the presence of food in the esophagus (and presumably stomach) of the LDS mice might have been interfering with absorption of the peanut protein and cholera toxin (compared to WT mice), which would affect our ability to sensitize the mice. We therefore modified our protocol to remove food from the mice for 10-12 hours prior to each sensitization dose to ensure the esophagus/stomach were empty at the time the dose of peanut/cholera toxin was delivered. As shown in Figure 1, using this modified protocol, LDS mice appear to demonstrate more severe signs of anaphylaxis (as measured by drop in core body temperature) compared to their WT littermates. To determine whether those mice that exhibited anaphylaxis exhibited increased Th2 immune responses to peanut, we isolated splenocytes from each of the mice at the end of the experiment, and stimulated the cells with peanut protein. Levels of IL-13 in culture supernatants were then assayed by ELISA. As shown in Figure 2, splenocytes from all mice that demonstrated a pronounced drop in rectal temperature exhibited robust Th2 cytokine responses to peanut. These experiments are currently being replicated, and additional experiments are being performed to look at peanut-specific IgE and IgG2a levels.

While our data suggest that LDS mice are more susceptible to developing food allergic reactions, we next wanted to ask the question of whether LDS mice are more likely to become sensitized to allergen, or whether they have increased effector responses independent of the allergen sensitization phase, or both. To address this question, we used a previously described model of passive systemic anaphylaxis, in which mice are administered allergen-specific IgE through the tail vein, followed by allergen challenge 24 hours later.² This approach allows us to essentially bypass the sensitization phase, so we can evaluate effector responses independent of sensitization. As shown in Figure 3, LDS mice showed a greater anaphylactic response than their WT littermates in this model, suggesting they possess more robust effector responses in anaphylactic reactions. While clinical scores were ascertained for both these experiments as well as the peanut allergy model described above, measurements of rectal temperature proved to be a more sensitive and reproducible measure of anaphylactic reactions.

TASK 2: Determine the efficacy of losartan in reducing the development and/or severity of allergic disease (eosinophilic esophagitis and susceptibility to peanut allergy) in LDS mice.

Our initial progress on this task was hampered by several factors, including refusal of the LDS mice to drink losartan in their drinking water. We attributed this issue to the fact that these mice develop severe eosinophilic esophagitis and esophageal dilatation/tortuosity. Indeed, this problem appeared to be specific for our LDS mice, as we have not encountered this issue with any of our other strains of mice that have been treated with losartan. After much trial and error, we have now solved this issue by adding sucrose and commercially available flavor additives to the water containing losartan (these same additives are also added to the mice drinking placebo water). The second issue that has slowed our progress on this task is that only about 20% of mice from an LDS mating (mouse heterozygous for LDS mutation mated to WT mouse) carry the LDS mutation as opposed to the expected 50%, suggesting there is some selection against/early death for mice harboring the LDS mutant allele. We have therefore needed to genotype more mice to obtain an adequate number for our trials. Active trials of losartan in our LDS mice are currently underway. We expect results within the next one to two months.

KEY RESEARCH ACCOMPLISHMENTS

- We have successfully induced peanut allergy in SV129 line of mice
- We have demonstrated that LDS mice manifest more severe anaphylactic reactions in a model of passive systemic anaphylaxis
- We have demonstrated that LDS mice likely manifest more severe systemic anaphylactic reactions in a murine model of peanut allergy, and that anaphylaxis is associated with increased Th2 responses (IL-13 expression) in response to peanut
- We have demonstrated that LDS mice spontaneously develop eosinophilic esophagitis
- We have initiated trials of losartan in the LDS mice

REPORTABLE OUTCOMES

None to report

CONCLUSIONS

Mutations in the genes encoding the receptor for TGF β that lead to Loeys Dietz Syndrome confer an increased susceptibility to develop IgE-mediated anaphylaxis to food proteins. These data have been replicated in LDS mice, and therefore support a prominent role for TGF β in the clinical manifestations of food allergy. LDS mice also spontaneously develop eosinophilic esophagitis (EE), a condition characterized by eosinophilic inflammation in the esophagus that is often caused by an abnormal immune response to food proteins, suggesting these mutations are sufficient to predispose to the development of allergic disease.

The monogenic nature of LDS suggests that mutations in a single gene can influence susceptibility to food-induced anaphylaxis and eosinophilic esophagitis (EE), suggesting this pathway may be an attractive therapeutic target. Since no treatment for food allergy is currently available, the identification of specific molecular targets that drive the development and/or severity of this disease are critically needed. Towards this end, we have ongoing trials to test the efficacy of losartan, an angiotensin II receptor antagonist with known efficacy in disorders associated with excessive TGF β signaling, in preventing and/or reducing the severity of allergic reactions (both food-induced anaphylaxis and EE) in mice with LDS. Losartan is already FDA-approved, including for use in children. If losartan proves to be efficacious in our mouse model, then human trials could follow quickly.

REFERENCES

1. Sun J, et al. *Journal of Immunology*. 2007;179(10):6696-6703.
2. Tilley SL, et al. *The Journal of Clinical Investigation*. 2000;105(3):361-367.

APPENDIX

No documents to report.

Figure 1

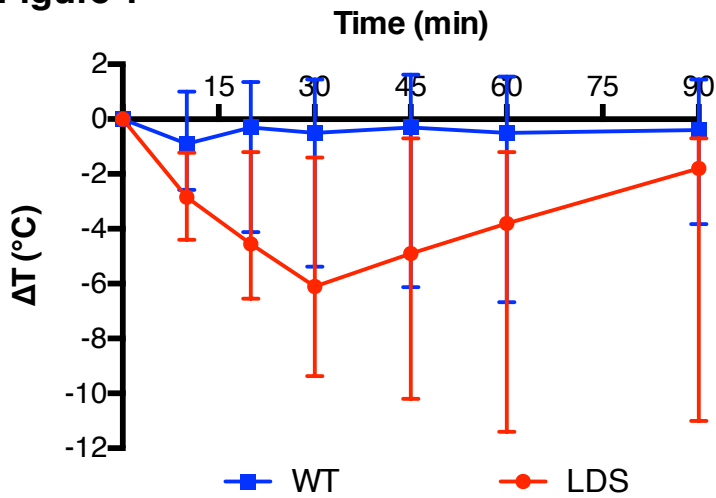


Figure 1. LDS mice exhibit more severe signs of anaphylaxis compared to WT littermates in a model of peanut allergy. LDS mice and WT littermates were sensitized with peanut and cholera toxin by oral gavage weekly for a total of four sensitization doses. Two weeks after the last sensitization dose was administered, mice were challenged intraperitoneally with peanut protein. Changes in rectal temperature (ΔT , °C), relative to baseline, were measured at each of the time points indicated following allergen challenge.

Figure 2

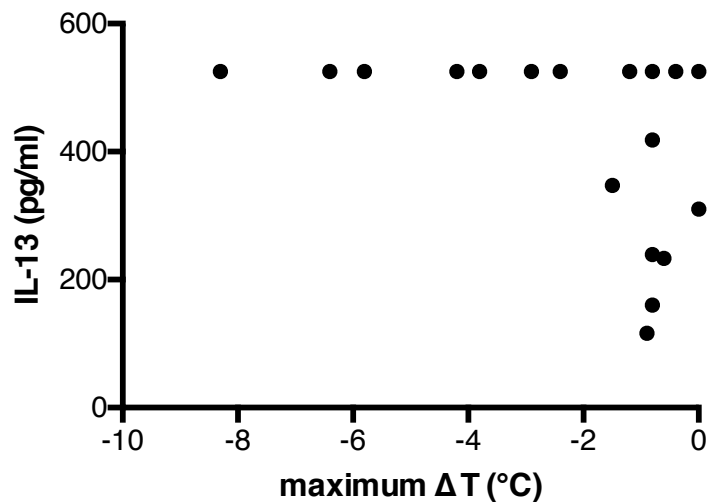


Figure 2. Peanut-induced IL-13 expression by splenocytes correlates with drop in rectal temperature following peanut challenge. LDS mice and WT littermates were sensitized and challenged with peanut. Splenocytes were obtained 90 minutes following the challenge, and stimulated overnight with peanut (200 μ g/mL) to induce cytokine expression. Level of IL-13 (pg/mL) in culture supernatants were measured by ELISA after overnight culture. Splenocytes from mice that exhibited more severe signs of anaphylaxis (greater drop in rectal temperature, ΔT , °C) generally expressed higher levels of IL-13 than those mice that experienced milder symptoms.

Figure 3

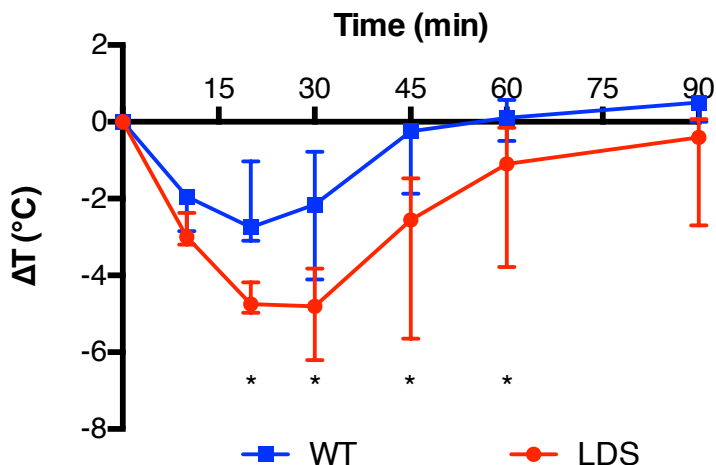


Figure 3. LDS mice exhibit more severe signs of anaphylaxis compared to WT littermates in a model of passive systemic anaphylaxis. LDS mice and WT littermates were administered 20 μ g of monoclonal anti-DNP IgE via tail vein injection. Twenty-four hours later, mice were challenged with 1 mg of DNP-HSA antigen via tail vein injection, and rectal temperatures were recorded as described above.